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ORIGINAL ARTICLE

MMP-7, -8, -9, E-cadherin, and beta-catenin expression in 34 ameloblastoma cases

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Abstract

Objectives: Ameloblastoma is a benign, locally aggressive odontogenic tumor with high recurrence rates. Matrix metalloproteinases (MMPs) mediate extracellular integrity in normal and pathological conditions, and exert multiple functions coordinating inflammation and tumor progression. E-cadherin and beta-catenin are adherence junction molecules in cell-to-cell connections. We investigated the involvement of MMP-7, -8, -9, E-cadherin, and beta-catenin in ameloblastoma and the surrounding extracellular matrix.

Material and methods: Our material consisted of 30–34 tissue samples from ameloblastoma patients of Helsinki University Hospital. We used immunohistochemistry to detect the expression of the biomarkers. Two oral pathologists independently scored the immunoexpression intensities and statistical calculations were made based on the results.

Results: E-cadherin expression was weaker in the maxillary than in mandibular ameloblastomas. Beta-catenin was expressed in the ameloblastoma cell membranes. We detected MMP-8 and -9 expression in polymorphonuclear neutrophils in the extracellular area and these MMPs correlated positively with each other. Osteoclasts lining bone margins and multinuclear giant cells expressed MMP-9. Neither MMP-8 nor MMP-9 immunoexpression could be detected in ameloblastoma cells. MMP-7 expression was seen in some apoptotic cells.

Conclusion: The fact that E-cadherin immunoexpression was weaker in maxillary compared to mandibular ameloblastomas might associate to earlier recurrences. It promotes the idea of mandibular and maxillary ameloblastoma exerting differences in their biologies. We detected MMP-8 and -9 in polymorphonuclear neutrophils which relates to these MMPs participating in extracellular remodeling through a mild inflammatory process. Bone degradation around ameloblastoma may be due to MMP-9 in osteoclasts but this phenomenon might be an independent process and needs further investigations.

KEYWORDS

ameloblastoma, beta-catenin, E-cadherin, MMP-7, MMP-8, MMP-9

1 | INTRODUCTION

Ameloblastomas are the most common odontogenic tumors with an estimated annual incidence of 0.5/million inhabitants. They are benign but locally aggressive odontogenic epithelial tumors located to the dentoalveolar region presenting as an intraosseous or peripheral lesion. Patients are typically 30–40 years old, though tumors occur in all age groups (El-Naggar, Chan, Grandis, Takata, & Slootweg, 2017). Recurrences develop in 20–93% of cases supposedly depending on the treatment modality (Neagu, et al., 2019). Pathogenesis is currently based on BRAF and SMO mutations in the mitogen-activated protein kinase (MAPK) and the Sonic Hedgehog signaling (SSH) pathways, respectively (Sweeney, et al., 2014). A growing interest focuses on the extracellular functions and inflammatory activities of the tumor environment. Still, the exact tumorigenesis remains to be resolved.

Matrix metalloproteinases (MMP) are a heterogeneous group of zinc-dependent, genetically distinct but structurally related proteinases responsible for the degradation and synthesis control of the extracellular matrix (ECM) and the basement membrane (BM). MMP's also participate by processing nonmatrix bioactive substrates involved in the membrane shedding, chemokine, or growth factor modification, and in regulating the activity of other proteases. They play an important role as the effective regulators of cell proliferation and differentiation, tissue homeostasis, and immune response (Löffek, Schilling, & Franzke, 2011).

The gelatinase MMP-9, when activated can break down type IV collagen and gelatin, the main elements of the ECM and BM (Roomi, Monterrey, Kalinovsky, Rath, & Niedzwiecki, 2009). MMP-9 plays a crucial role in tumor progression, from angiogenesis, to stromal and bone remodeling, and ultimately to metastasis (Farina & Mackay, 2014). Various studies confirm and evidence the presence of MMP-9 in ameloblastoma cells and the ECM and BM processings (Anne, Krisnuhoni, Chotimah, & Latief, 2014; Kumamoto, Yamauchi, Yoshida, & Ooya, 2003; Ribeiro et al., 2009; Yang, et al., 2018).

MMP-8 or collagenase-2 is a neutrophil derived collagenase with a multifunctional role in mediating inflammation, and inhibiting cancer invasion and metastasis. Its protective nature seems to depend on the tissue of origin (Juurikka, Butler, Salo, Nyberg, & Åström, 2019). It degrades efficiently type 1 collagen among other ECM and non-ECM substrates. MMP-8 is thought to play a role in inflammation and different tumor processes (Juurikka et al., 2019). To our knowledge, there is no previous studies of the MMP-8 expression in ameloblastomas.

MMP-7, known as matrilysin, is found constitutively in many epithelial cell types, especially ductal cells of exocrine glands like salivary glands, liver, breast and colon (Saarialho-Kere, Crouch, & Parks, 1995). It has a function in tumor invasion, metastasis and as a pro-MMP-2, -8 and -9 activator. It has also been associated to angiogenesis in normal physiological processes as well as in cancer progression (Nishizuka et al., 2001)

Adherens junctions anchor epithelial cells together and mediate cell and tissue behavior via transmembrane cadherin/catenin-based complexes bound to intracellular microfilaments. These structures contribute to the formation of solid tissue and coordinate intra- and intercellular signaling (Niessen, 2007). E-cadherin is a Ca-dependent transmembrane protein which is linked intracellularly to p120-, α-, β-, and γ-catenins (Tian, Liu, Niu, et al., 2011). E-cadherin/beta-catenin complexes modulate Wnt signaling and are involved in epithelial to mesenchymal (EMT) and mesenchymal to epithelial (MET) transitions, which are essential in embryo development, tissue fibrosis, and cancer progression. Mediators of inflammation, including MMPs, growth factors, and cytokines may cause dysregulation and loosening of this adherence complex (Shang, Hua, & Hu, 2017). Via transactivation of target genes involved, the nuclear accumulation of beta-catenin promotes tumor progression and proliferation (Brabletz, Jung, Dag, Hlubek, & Kirchner, 1999) (Figure 1). Beta-catenin can regulate the expression of the MMP-7 in human colorectal cancer (Brabletz et al., 1999).

In this study, we explored the expression of MMP-7, -8, -9, beta-catenin, E-cadherin in the ECM and ameloblastoma tumor cells and evaluated the role of those markers combined with clinical factors in predicting recurrence of ameloblastoma.

2 | MATERIAL AND METHODS

2.1 | Patient material

Our series consisted of 34 ameloblastoma patients treated at the Helsinki University Hospital, Department of Oral and Maxillofacial Diseases (HUCH). The Department of Pathology (HUSLAB) archives provided us with the formalin-fixed paraffin-embedded tissue samples. We have described the patient material in more detail in our previous study (Kelppe, et al., 2019). Since some samples were no longer available, 8 cases with recurrent ameloblastoma of which we had no primary tumor tissue available for our preliminary studies were added to the series. The Ethics Committee of Surgery and HUCH's Internal Review Board (Dnro 151/13/03/02/2015) granted their approval for this study.

2.2 | Immunohistochemistry

For immunohistochemistry, we used 3 μm thick formalin-fixed paraffin-embedded tissue sections, which we attached on the glass in 60°C for 1–2 hr. The samples underwent deparaffinization in xylene. A graded alcohol series to water rehydrated the tissue. A heated buffer (Dako ENvision Flex) specific for each antibody functioned as a heat induced epitope retrieval. For staining, we used Autostainer 480 (Labvision UK Ltd., Suffolk, UK) with Dako REAL EnVision Detection System, Peroxidase, Rabbit/Mouse (Dako, Glostrup, Denmark). The primary incubation of each antibody was for 1 hr in +4°C and a secondary HRP-coupled antibody for half an hour. To visualize results we used either a DAB (brown) or magenta (pink) chromogen. A hematoxylin incubation for 2 minutes counterstained the nuclei. A graded alcohol series dehydrated the tissue. The samples were finally mounted. Primary antibodies were: anti-MMP-7 (1:1000, EMD Millipore Corporation, Temecula, CA), anti-MMP-8 (1:400), anti- MMP-9 (1:1000, NeoMarkers, Fremont CA and Calbiochem Inc., San Diego,

CA), antibeta-catenin (1:400, Thermo Fisher Scientific MA), and ant-E-cadherin (1:100 Thermo Fisher Scientific MA). Colon and oral squa-

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